MÖSSBAUER SPECTROSCOPY OF IRON-CONTAINING DERMAL GRANULES FROM *MOLPADIA INTERMEDIA*

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Dermal granules containing hydrous ferric oxide cores from *Molpadia intermedia* were studied by Mössbauer spectroscopy from 1.5 to 300 K and in magnetic fields up to 80 kOersted at 4.2 K. A magnetic phase transition to an antiferromagnetically ordered state is observed at 10 K. The results are compared with the magnetic behavior of micellar cores of ferritin from eukaryotes and iron-storage materials from prokaryotes.

In addition to the utilization of iron atoms in proteins for oxygen and electron transport and other metabolic purposes, many organisms sequester iron in the form of hydrous ferric oxides [1]. The most widely studied biomineralization product of this type occurs in the iron storage protein ferritin, where it forms a 70 Å micellar core of approximate composition $(\text{FeOOH})_8 \cdot \text{FeO} \cdot \text{OPO}_3\text{H}_2$ [2,3] or $2.5 \text{ Fe}_2\text{O}_3 \cdot 4.5 \text{ H}_2\text{O}$ [1,4] surrounded by a polypeptide sheath. Micelles of similar composition occur in the related iron-storage material hemosiderin [5] and in bacterioferritin from *Azotobacter vinelandii* [6]. Magnetite (Fe_3O_4) is the most common of the known iron oxide biominerals [7]. It occurs in chitons [8–10], magnetotactic bacteria [11] and a variety of other organisms [12]. Ferritin forms the precursor mineral of magnetite in chitons [9,12]. A storage material with a Mössbauer spectrum similar to that of ferritin is observed in magnetotactic bacteria [11]. Another iron-rich storage material of as yet unknown composition has been observed in prokaryotic cells grown in iron-rich media [13–15].

Molpadia intermedia (Holothuroidea) is a species of marine invertebrates commonly known as sea

cucumber. Starting at the late juvenile stage, these organisms synthesize iron- and phosphate-rich dermal granules ranging in size from 10 to 350 µm which serve as strengthening agents in the connective tissues of their dermis [16]. The microarchitecture and mineralogic composition of the granules have been studied by a variety of physical and chemical techniques [17]. They consist of layers composed of two types of spherical to ellipsoidal subunits, 0.03–0.24 µm in diameter, separated and alternately encapsulated by organic material. One type of subunit contains water, iron and phosphate, with lesser amounts of calcium and magnesium. These deposits are X-ray amorphous and in turn consist of electron-dense subunits 90–140 Å in diameter. The iron is present in the form of hydrous ferric polymeric units similar to the iron-containing micelles of ferritin.

Because the iron-containing granular cores from *Molpadia* are a structurally and chemically well characterized biomineralization product, it is interesting to investigate their magnetic properties for comparison with the iron micelles of ferritin and with the other iron-storage materials from prokaryotic cells, as well as with the precursors to magnetite formation in magnetotactic bacteria and chitons. Therefore, we have made Mössbauer spectroscopic measurements on the isolated dermal granules from 1.5 to 300 K

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and in an external magnetic field of 80 kOersted at 4.2 K.

Experimental Procedure

Granules were extracted from the dermal tissues of *M. intermedia* s.l. as described by Lowenstam and Rossman [17]. Mössbauer measurements were made using a conventional, constant-acceleration spectrometer with a source of ^{57}Co in rhodium, which was maintained at room temperature. The sample was mounted on a copper block inside a Janis Veritemp Dewar and temperatures between 1.5 and 300 K were maintained and measured with a calibrated silicon diode and a Lake Shore Cryotronics Temperature Controller. The spectra were least-squares fitted by a program developed by J. Teillet and F. Varret (personal communication) to generate theoretical Mössbauer spectra with Lorentzian lineshapes in order to yield isomer shifts, quadrupole splittings, internal magnetic fields, linewidths and line intensities.

Spectra of *Molpadia* dermal granules were obtained between 1.5 and 300 K and in a longitudinal magnetic field of 80 kOersted at 4.2 K. Spectra of lyophilized horse spleen ferritin and lyophilized iron-storage material from *Escherichia coli* prepared as previously reported [13] were also obtained at several temperatures between 4.2 and 300 K.

Results

The spectrum of *Molpadia* dermal granules at temperatures between 10 and 300 K is a broadened quadrupole doublet with an average linewidth $\Gamma = 0.55 \pm 0.02$ mm/s, indicating a distribution of electric-field gradients at the iron sites. Satisfactory fits were obtained with two overlapping quadrupole doublets simulating the distribution in the electric-field gradients. The weighted averages of the Mössbauer parameters at various temperatures are listed in Table I. Below 10 K the spectrum broadens and magnetic hyperfine structure appears, with the effective magnetic field at the nucleus increasing with decreasing temperature (Fig. 1). The breadth of the outer lines compared to the inner lines in Fig. 1c indicates a distribution of magnetic hyperfine fields. The magnetically split spectra were fitted by assum-

TABLE I.

MÖSSBAUER PARAMETERS

Weighted averages of the two superimposed subspectra least-square fits are presented. Isomer shifts (δ) are relative to metallic iron at room temperature.

Compound	T (K)	δ (mm/s)	ΔE_Q (mm/s)
<i>Molpadia</i> dermal granules	10.4	0.51 ± 0.02	0.86 ± 0.03
	20	0.50	0.83
	130	0.48	0.84
	150	0.46	0.83
	200	0.44	0.81
	250	0.41	0.80
<i>E. coli</i> storage material	300	0.39	0.83
	45	0.52	0.68
	150	0.50	0.66
	200	0.46	0.66
	250	0.44	0.65
Ferritin	300	0.41	0.64
	130	0.47	0.73
	200	0.42	0.77
	250	0.40	0.77
Amorphous ferric gel	300	0.37	0.72
	77	0.47 a,b	0.81 a

a Ref. 18.

b 0.62 mm/s quoted relative to Cr source in Ref. 18.

ing 30 overlapping subspectra with different magnetic hyperfine fields between 0 and 500 kOersted relative intensities as free parameters. The smooth results of these fits at several temperatures are presented in Fig. 2, where relative intensity is plotted as a function of effective magnetic field at the nucleus. At 1.6 K, the mode of the distribution is at 160 kOersted and moves to progressively lower field with increasing temperature, collapsing at about 10 K to a quadrupole doublet and the magnetically split subspectra coexist from approx. 8.0 to 10.0 K. The relative intensity of the quadrupole doublet is given in Fig. 2. The collapse of the magnetic hyperfine splitting is indicative of a magnetic transition at about 10 K with a distribution of transition temperatures in the sample from 8.0 to 10.0 K.

The spectrum obtained in a longitudinal magnetic field at 80 kOersted at 4.2 K is shown in Fig. 3. The effect of the applied field is to broaden the spectrum without substantially changing the line positions.

Fig. 1. Mössbauer spectra of *Molpadia* dermal granules at (a) $T = 20$ K, (b) $T = 7.2$ K and (c) $T = 1.6$ K.

Fig. 2. Distribution of magnetic hyperfine fields at the iron nucleus in *Molpadia* dermal granules at different temperatures obtained from least-squares fits of the Mössbauer spectra. The numbers in parentheses give the percentage of absorption area under the central quadrupole doublet (Q.D.).

Fig. 3. Mössbauer spectra of *Molpadia* dermal granules at $T = 4.2$ K: (a) in zero field and (b) in an applied field, $H = 80$ kOersted parallel to the γ -ray direction.

Fig. 4. Isomer shifts (relative to metallic iron) for *Molpadia* dermal granules (●), *E. coli* storage materials (△), and ferritin (X) as a function of temperature.

relative intensities compared to zero field (Fig. 3a). This result is expected for a randomly oriented powder sample in which the spins are coupled antiferromagnetically or ferromagnetically [18], with anisotropy fields $H_A \gg 80$ kOersted.

Spectra for horse spleen ferritin and *E. coli* storage material were similar to previously reported spectra in Refs. 19, 20 and 13, respectively. Computer fits were obtained assuming two overlapping quadrupole doublets in each case. Representative data are pre-

Fig. 5. Relative absorption intensity of the Mössbauer spectrum for *Molpadia* dermal granules (●), *E. coli* storage protein (△) and ferritin (X) as a function of temperature.

sented in Table I. Isomer shifts and relative intensities obtained from the fits are plotted in Fig. 4 and 5. Again, only the weighted averages of the Mössbauer parameters of the two superimposed subspectra are presented. Also presented in Table I are parameters for a naturally occurring amorphous ferric gel of approximate composition $\text{Fe}(\text{OH})_3 \cdot 0.9 \text{H}_2\text{O}$ [18].

Discussion

The *Molpadia* spectra can be interpreted in terms of a transition from a paramagnetic state to a magnetically ordered state below 10 K. The distribution of transition temperatures below 10 K in the sample as well as the distribution of hyperfine fields in the ordered state are consistent with the amorphous nature of the iron-containing subunits, as determined by X-ray analysis [17]. In an amorphous structure the iron-iron distances and the density of iron atoms might be expected to vary, giving rise to a distribution of the Néel temperatures and hyperfine fields. However, spectra with a superposition of magnetically split and paramagnetic components near the Néel temperature have also been obtained in well-crystallized $\gamma\text{-FeOOH}$ [21].

The high-temperature spectra of the iron-rich cores of *Molpadia* dermal granules, ferritin and *E. coli* storage material are very similar, but there are experimentally distinguishable differences in the quadrupole splittings and isomer shifts. The observed quadrupole splittings for *Molpadia* are on the average about 10% higher than those observed for ferritin and about 20% higher than those of *E. coli* storage protein (Table I). The isomer shifts of the various materials are similar to the *Molpadia* values, falling between those of *E. coli* and ferritin (Fig. 3). The major differences, however, are in the temperature dependence of the respective spectra. The Mössbauer spectrum of ferritin and its insoluble analogue hemosiderin shows a temperature dependence characteristic of superparamagnetic behavior of magnetically ordered fine particles [19,20]. That is, although the iron core is antiferromagnetically ordered below about 200 K [19], thermally activated transitions between equivalent easy axes motionally narrow the magnetic hyperfine spectrum to a quadrupole doublet when the transition rate exceeds the Larmor precession time. Thus, the high-temperature spectrum ($T >$

50 K) is a broadened quadrupole doublet, with isomer shift and quadrupole splitting characteristic of high-spin Fe^{3+} . The low-temperature spectrum ($T < 10$ K) is a magnetic hyperfine sextet with a well defined magnetic splitting of 497 kOersted independent of temperature. As the transition rate is an exponential function of the temperature, magnetic anisotropy and volume of the micelles [22], a distribution of micellular diameters about the 70 Å mean results in a coexistence of quadrupole and magnetic hyperfine spectra for $10 < T < 50$ K, with the relative intensity of the high-temperature spectrum increasing with increasing temperature. The splitting of the low-temperature spectrum is also independent of temperature in this temperature range ($10 \text{ K} < T < 50 \text{ K}$). Similar spectra with a low-temperature magnetic splitting of 458 kOersted and a coexistence region $10 < T < 20 \text{ K}$ are observed for the amorphous ferric gel presented in Table I [18].

The Mössbauer spectrum for $T > 10 \text{ K}$ of the iron-storage material from *E. coli* [13] and the other prokaryotes *Proteus mirabilis* [15], and *Mycoplasma capricolum* [14], is a quadrupole doublet with parameters characteristic of high-spin Fe^{3+} . A six-line magnetic hyperfine spectrum with an effective magnetic field at the nucleus of 430 kOersted is observed at $T < 1 \text{ K}$. Above 1 K the lines broaden and the splitting decreases with increasing T and collapses into the quadrupole doublet at about 3.5 K. Between 1.2 and 3.5 K the doublet and sextet are superposed, indicating a spread of magnetic transition temperatures.

The low-temperature magnetic behavior of the *Molpatria* dermal granules as observed via Mössbauer spectroscopy is more similar to that of the prokaryotic iron-storage materials than to ferritin. Firstly, there is a low-temperature ($T_c \approx 10 \text{ K}$) magnetic transition with a distribution of transition temperatures ($\Delta T_c \approx 2 \text{ K}$). In the prokaryotic storage materials the magnetic transition occurs at about 3 K, whereas in ferritin the magnetic transition occurs at approx. 200 K [19]. Secondly, there is a distribution of saturation hyperfine fields that peaks at 440 kOersted. In the prokaryotic storage material the saturation hyperfine fields peak at 430 kOersted [13], whereas in ferritin the saturation hyperfine field distribution peaks at 497 kOersted [19].

These differences may reflect different biological

functions of the materials. Iron storage in ferritin is reversible and Fe ions can be extracted from the micelles in response to metabolic demands for iron. This reversible storage may require a high degree of structural order in micelles [23]. Heald et al. [3] have proposed a model in which the Fe ions are bound in planar sheets, with the sheets relatively well separated from each other and terminated by H_2PO_3 . By contrast, iron storage in the dermal granules of *Molpatria* appears to be largely a final biological product and in the main is presumably not remobilized. Here, the hydrous iron oxide core is X-ray amorphous [17] and has a considerably higher concentration of PO_3 relative to Fe than in ferritin. In this material the iron atoms may be further apart on the average with a consequent reduction in the magnetic ordering temperature. If the magnetic properties are indicative of biological function, this would suggest that the prokaryotic ion-storage material [13] is a final product and not a reversible iron-storage location.

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